

Prediction of Avian Repellency from Chemical Structure: The Aversiveness of Vanillin, Vanillyl Alcohol, and Veratryl Alcohol

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The effectiveness of bird repellents is associated with the presence of an electron-withdrawing group (carbonyl or carboxyl) and an electron-donating group in resonance on a phenyl ring. The present experiments were designed to examine the relative importance of these structural features. European starlings (*Sturnus vulgaris*) were presented with vanillin, vanillyl alcohol, and veratryl alcohol in two-cup and one-cup feeding trials and in one-bottle drinking tests. In feeding trials, veratryl alcohol was significantly more aversive than the other two chemicals. In drinking tests, veratryl alcohol was repellent only at the highest concentration (0.5% ml/ml), and was lethal at that concentration and at 0.1 and 0.05% ml/ml. Together, the findings have several implications. From a basic perspective, the data emphasize the importance of electron-donating groups on the phenyl ring of repellent chemicals. From the practical perspective, the data suggest veratryl alcohol as an avian toxicant, and warn against generalization from feeding to drinking tests. We propose that avian repellents must be tailored to the specialized settings in which they are used. © 1991 Academic Press, Inc.

INTRODUCTION

Few nonlethal chemicals (i.e., repellents) are available for the control of avian depredation and nuisance problems (1). No chemicals are commercially available to prevent the accidental ingestion of pelleted agricultural chemicals, treated seeds, agricultural wastewater, or the toxic solutions found in industrial evaporating ponds. We are attempting to develop repellents for these diverse applications, and consequently are exploring relationships between ingestive behavior and chemical structure.

The available data indicate that anthranilate derivatives (i.e., aminobenzoates) (2, 3) are effective bird repellents at concentrations between 1.0% (in feed) and 0.03% (in aqueous solution). The aversiveness of these substances is chemosensory in nature (i.e., olfaction, chemesthesis (4)), and is associated with two physicochemical parameters: (a) the presence of a hydrogen-bonded (H-bonded) ring in association with the phenyl ring, and (b) substitution pattern

on the phenyl ring (5). Between these parameters, the latter appears relatively more important (3). For example, methoxyl substitution at the *ortho* position of acetophenones still yields repellency, yet this molecule is incapable of forming intramolecular hydrogen bonds (Fig. 1). Similarly, substitution at the *para* and *meta* positions still result in repellency, yet these isomers are incapable of forming H-bonds. In contrast, hydroxyl substitution at the *ortho* position of acetophenones, which allows intramolecular hydrogen bonding, produces a weaker repellent than any of the isomeric methoxyl or amino substituent counterparts. Furthermore, solvation effects would eliminate the repellency of substances in water if H-bonded rings were essential—yet many substances are most effective in aqueous solution.

The present experiments were designed to further explore the relationship between avian repellency and electron-withdrawing and electron-donating groups. Vanillin, vanillyl alcohol, and veratryl alcohol were

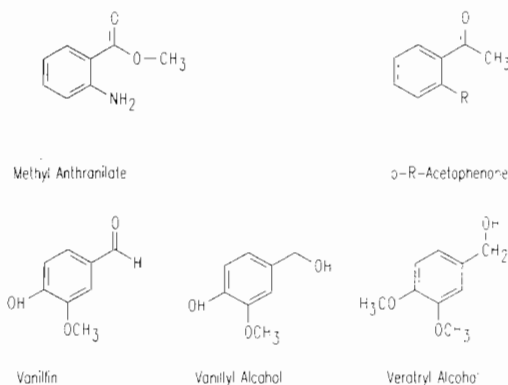


FIG. 1. Chemical structures of acetophenone derivatives and vanillin, vanillyl alcohol, and veratryl alcohol.

chosen as stimuli because they permitted us to examine the roles played by these groups and their contribution to resonance in both feeding and drinking, in the absence of a confounding H-bonded ring at the *ortho* position (Fig. 1). In vanillin, the electron-withdrawing group is in resonance with the hydroxyl group. This hydroxyl group and the methoxyl group form a five-membered H-bonded ring. Vanillyl alcohol entirely eliminates the electron withdrawal by resonance. Thus, comparison of consumption data for vanillin and vanillyl alcohol would show the importance of the electron-withdrawing group. Examination of veratryl alcohol would show the role of electron-donating groups and the importance of the H-bonded ring for repellency because it has neither an electron-withdrawing carbonyl group nor an H-bonded ring. Instead, it has a methoxyl group (rather than hydroxyl group), resulting in increased electron-donation to the phenyl ring. Finally, comparison of results from the present experiment with those collected for acetophenones, namely, amino, hydroxy, and methoxy derivatives (3), could reveal the contribution of the amino group versus that of the methoxyl group to repellency.

Although we had no *a priori* evidence to suspect that vanillin, vanillyl alcohol, or veratryl alcohol would be bird repellent, these compounds are structurally similar to co-

niferyl alcohol and dimethoxycinnamyl alcohol; both of which are repellent to birds (6). Further, coniferyl alcohol is a precursor to vanillin and its derivatives in their biosynthetic (shikimic acid) pathway (7).

MATERIALS AND METHODS

Experiment 1

Subjects. European starlings were decoy-trapped (8) at Sandusky, Ohio, and brought to the laboratory. Upon arrival, each bird was individually caged ($61 \times 36 \times 41$ cm) in a room with a 12:12 light:dark cycle and an ambient temperature of 23°C. Free access to tapwater and feed (Purina Flight Bird Conditioner, Purina Mills, St. Louis, MO) was permitted during the 2-week adaptation period before experiments began.

Chemicals. Reagent grade vanillin (CAS 121-33-5), vanillyl alcohol (CAS 498-00-0), and veratryl alcohol (CAS 93-03-8) were purchased from Aldrich Chemical Co. (Milwaukee, WI). To produce test samples, the chemicals were dissolved in ether, and then mixed with feed (250 ml of ether/250 of feed). After mixing, the ether was evaporated by placing treated feed samples under a hood for 24 hr, and stirring occasionally. Ether was used as the solvent because vanillin, vanillyl alcohol, and veratryl alcohol are not readily soluble in water. In addition, the high volatility of ether reduced the chance that trace amounts would remain as contaminants in feed samples after drying. Three equimolar concentrations of each chemical were prepared. These were: (a) vanillin, 1.713 (g/g), 0.856, 0.214%; (b) vanillyl alcohol, 1.737, 0.869, 0.217%; and (c) veratryl alcohol, 1.895, 0.948, and 0.237%. All treated feed samples were stored in closed containers at -17°C until use.

Two-cup tests. During a 4-day pretreatment period, all birds were deprived of feed overnight. Within 2 hr of light onset, birds ($n = 36$) were presented with two cups, each containing 10 g of ether-treated feed.

After 2 hr, feed consumption was measured, maintenance diet (untreated feed) was returned to the cages, and the birds were left undisturbed until lights out. The food deprivation regime remained in effect throughout the treatment period described below.

During the 4-day treatment period that followed, birds were randomly assigned to one chemical ($n = 12$ birds/chemical), and one concentration ($n = 4$ birds/concentration) (Fig. 2). On each day, each bird was given 10 g of stimulus-treated diet in one cup and 10 g of untreated (ether only) diet in the other. After 2 hr, consumption was measured. Tapwater was provided *ad libitum*.

One-cup tests. Pretreatment and treatment trial procedures were identical to those described for two-cup tests, except that each bird was given only one cup containing untreated (pretreatment period) or treated (treatment period) feed daily (Fig. 2).

Both two-cup and one-cup tests were used because the former is more sensitive

for detecting avoidance, *per se*, while the latter is a better measure of absolute repellency. The same birds were used in both two-cup and one-cup tests so that we could examine the extent to which birds might habituate to stimulus presentations. The use of experienced birds in one-cup tests permitted evaluation of this possibility.

Analysis. Two-cup data were evaluated in a three-factor ANOVA with repeated measures between cups. One-cup data were assessed in a three-way ANOVA with repeated measures between periods. The independent factors in both analyses were chemicals (three levels) and concentrations (three levels). Tukey Honestly Significant Difference (HSD) tests (9) were used to isolate significant differences among means ($P < 0.05$).

Experiment 2

Veratryl alcohol was more effective than vanillin or vanillyl alcohol. Therefore, Experiment 2 was performed to evaluate the lowest concentration of veratryl alcohol that would repel birds. We used drinking

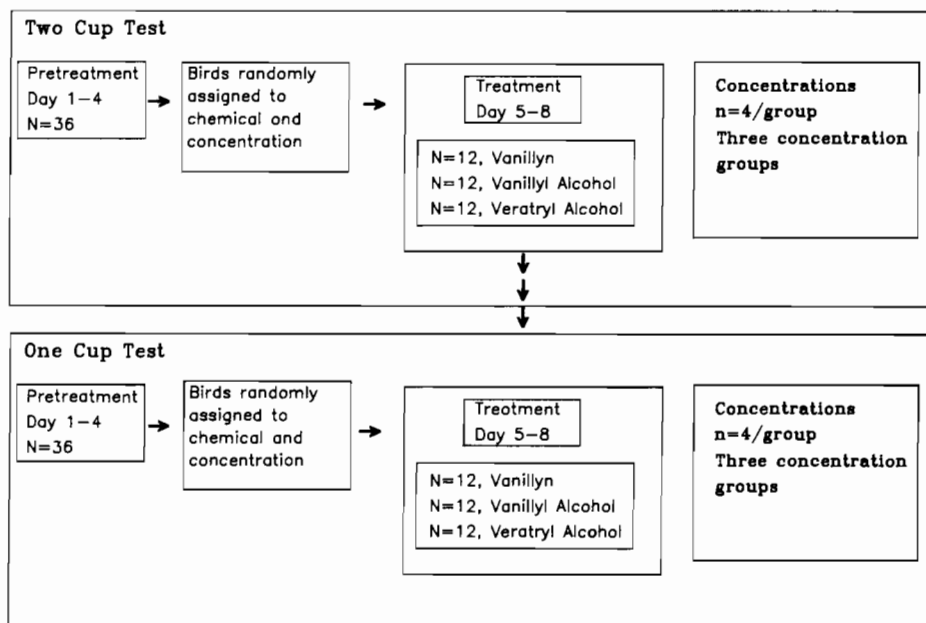


FIG. 2. Flow diagram of the procedures followed in Experiment 1. Concentrations of chemicals were equimolar, thus percentages (g/g) in diet are slightly different among substances.

trials rather than feeding trials in an attempt to maximize detection of the chemical.

Subjects. Thirty-six starlings were decoy-trapped at Sandusky, Ohio, and transported to the laboratory. Upon arrival, the birds were caged and maintained as previously described. Before experiments began, the birds were permitted free access to feed and tapwater.

Procedure. All birds were given 3 days of pretreatment during which water consumption was measured between 0930 and 1530 hr. Individuals whose variance about the 3-day mean was greater than ± 1 standard deviation of the population variance were excluded from subsequent testing. Those birds with stable daily water consumption were ranked according to mean water consumption and assigned to treatment groups. That bird with the highest water consumption was assigned to the first treatment group, the bird with the second highest consumption was assigned to the second group, and so forth, until all birds were assigned.

On the day of treatment, all birds were given a 6-hr drinking trial. Beginning at 0930, tapwater was replaced with aqueous solutions of veratryl alcohol, and consumption was recorded at 2-hr intervals. The chemical concentrations in solution were: 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001% (ml/ml). Afterward, all birds were given free access to tapwater. On the day following treatment, drinking was measured between 0930 and 1530 hr so that post-treatment and mean pretreatment drinking could be compared.

Analysis. Analysis of time periods failed to reveal significant differences. Only evaluation of the 6-hr measurements are reported here. A two-factor ANOVA with repeated measures among periods (pretreatment, treatment, post-treatment) was used to evaluate the data. The independent factor was groups (i.e., concentrations). Scheffe post hoc tests (9) were used to isolate significant differences among means ($P < 0.05$).

RESULTS

Experiment 1

Two-cup tests. There were significant differences among chemicals ($F = 5.4$; 2,27 df; $P < 0.01$). Overall, consumption by birds presented with veratryl alcohol ($\bar{X} \pm SE = 1.8 \pm 0.3$ g) was significantly lower than overall consumption by birds presented with vanillin (5.3 ± 0.4 g) or vanillyl alcohol (5.2 ± 0.4 g). Also, all groups ate less treated (4.1 ± 0.4 g) than control (5.3 ± 0.3 g) feed ($F = 181.8$; 1,27 df; $P < 0.00001$). Finally, there was a significant interaction between concentrations and cups ($F = 3.7$; 2,27 df; $P < 0.04$). Although less treated feed was consumed at all concentrations, the difference in consumption between treated and control feed was greatest at the highest concentration and least at the lowest concentration (Fig. 3).

One-cup tests. There were significant differences among chemicals ($F = 25.5$; 2,27 df; $P < 0.00001$). Birds presented with veratryl alcohol exhibited lower overall consumption (1.8 ± 0.5 g) than birds presented with vanillin (2.5 ± 0.3 g) or vanillyl alcohol (2.2 ± 0.5 g). Also, consumption was significantly higher during the pretreatment period (3.6 ± 0.4 g) than during the treatment period (0.7 ± 0.3 g) ($F = 85.4$; 1,27 df; $P < 0.00001$). Finally, there was an interaction between chemicals and periods ($F = 69.4$; 2,27 df; $P < 0.00001$). Post hoc examination of this effect indicated that veratryl alcohol was the only chemical to produce a significant drop in consumption between pretreatment and treatment (Fig. 4).

Experiment 2

There were significant differences among treatment ($F = 4.77$; 2,60 df; $P < 0.012$) and periods ($F = 2.2$; 10,60 df; $P < 0.03$). Post hoc tests did not reveal pretreatment differences among groups. However, there were differences among groups in drinking on the day of treatment day. Birds given the highest (0.5%) concentration of veratryl al-

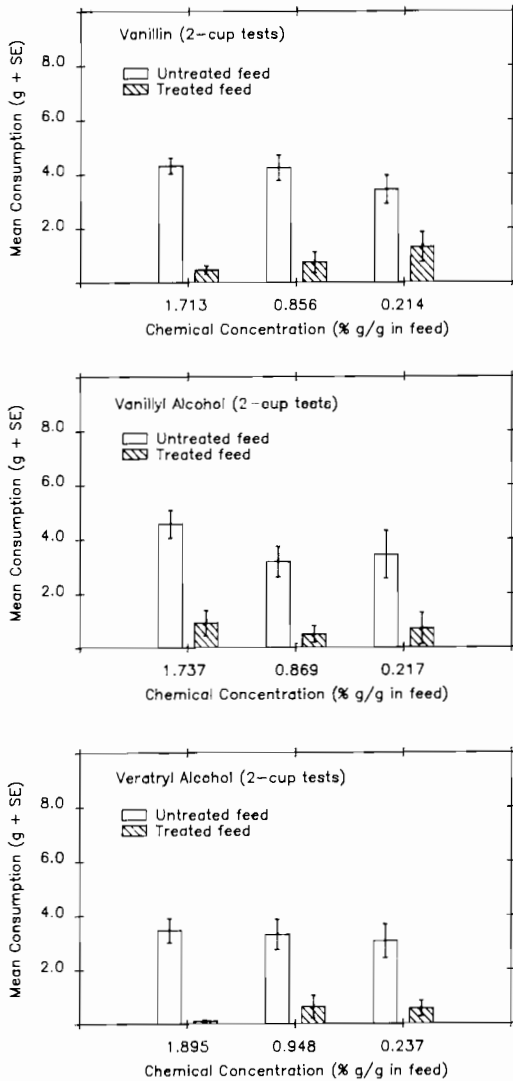


FIG. 3. Consumption by starlings of vanillin, vanillyl alcohol, and veratryl alcohol in two-cup feeding tests. Capped vertical bars represent standard errors of the means.

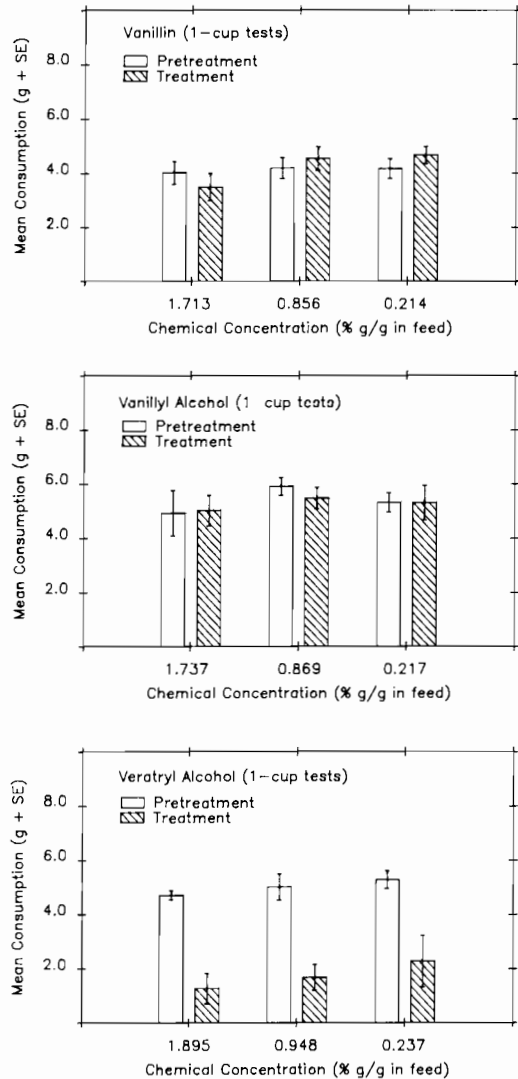


FIG. 4. Consumption by starlings of vanillin, vanillyl alcohol, and veratryl alcohol in one-cup feeding tests. Capped vertical bars represent standard errors of the means.

cohol significantly decreased water consumption (Fig. 5), and this decrease carried over into drinking on the post-treatment day. This result reflected the unexpected toxicity of the alcohol. Many of the birds given 0.5, 0.1, or 0.05% solutions were moribund on the post-treatment day. Within 24 hr, 4 of 6 birds died in the 0.5% group, 2 of 6 died in the 0.1% group, and 3 of 6 died in the 0.05% group.

DISCUSSION

Veratryl alcohol was significantly more repellent than either vanillin or vanillyl alcohol in both two-cup and one-cup feeding tests. These results are consistent with our earlier results (3) showing that the H-bonded ring is not required for repellency, although it may play an ancillary role. Had the ring structure been important, then both

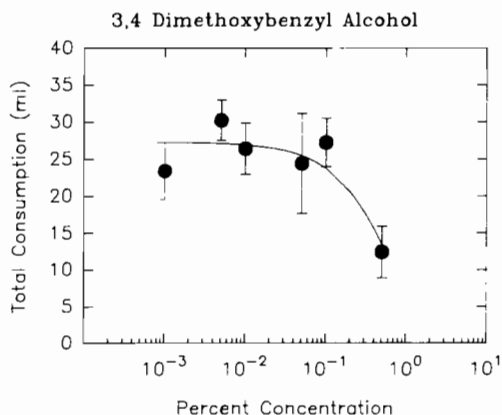


FIG. 5. Consumption by starlings of veratryl alcohol in one-bottle drinking tests. Capped vertical bars represent standard errors of the means. The curve is a least-squares fit for a nonlinear function of the form $y(x) = a(\exp(sx))$ using the Marquardt-Levenberg algorithm, where "a" was drinking at the 0% (pretreatment) concentration; "s," the rate of change in consumption as concentration increased; and "x" the concentration of chemical presented. This two-parameter model was used because it best fit the data when compared to other functions as determined by summation of residuals.

vanillyl alcohol and vanillin should have been more effective than veratryl alcohol.

Our results support and extend the hypothesis that increased electron donation is associated with repellency. Veratryl alcohol has the greatest capacity for electron donation, and was the most aversive stimulus. In addition, vanillin was less effective than vanillyl alcohol, suggesting that decreased electron withdrawal from the phenyl ring increases potency. Specifically, electron donation to the phenyl ring is the same for both of these compounds, but electron withdrawal by resonance cannot occur in vanillyl alcohol, making its phenyl ring more electron rich. These results suggest that an electron-withdrawing carbonyl group is not necessary for repellency, and that it may actually diminish effectiveness.

o-Aminoacetophenone is repellent in feeding trials identical with those reported here at concentrations as low as 0.1% (5), and in drinking trials at concentrations as low as 0.03% (3). Thus this acetophenone is

much more effective than veratryl alcohol, suggesting the importance of an amine group relative to other heteroatoms. The relative superiority of *o*-aminoacetophenone also suggests the importance of basic functionalities in the molecules. For example, the amino group in *o*-aminoacetophenone is more basic than the methoxyl group in veratryl alcohol, and basicity may underlie repellency. This observation is consistent with our results for hydroxy- and methoxyacetophenone in which the former, being relatively more acidic, was significantly less active than the latter (3).

The results of the drinking trials were unexpected. Although the highest concentration of veratryl alcohol was repellent relative to tapwater, it was not sufficiently aversive to prevent ingestion of a lethal dose. Intermediate concentrations were not repellent relative to tapwater, and again, sufficient quantities were consumed to cause death. Using mean consumption for birds presented with the 0.1 and 0.05% concentrations (27.2 ± 7.4 and 24.3 ± 15.2 ml, respectively), we estimate that the avian LD50 of this chemical in aqueous solution is between 4.3 and 2.3 ml/kg.

MANAGEMENT IMPLICATIONS

Vanillin, vanillyl alcohol and veratryl alcohol are not strongly aversive to birds, and we do not recommend them as potential avian repellents. However, useful management information is implied by the results. For example, differences between Experiments 1 and 2 suggest that candidate repellents should be evaluated in both feeding and drinking trials. Data collected in the former context may not generalize readily to the latter. In addition, veratryl alcohol was lethal but not repellent. We suggest that this substance might serve as an avian poison in situations where drinking can be used to deliver toxicant. Finally, the most important management implication of these experiments is that basicity via electron donation is correlated with bird activity. This

finding is consistent with two earlier reports (3, 5). We speculate that examination of existing agricultural chemicals for this molecular characteristic may yield new and readily available chemicals for pest and nuisance bird control.

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